

10/506877

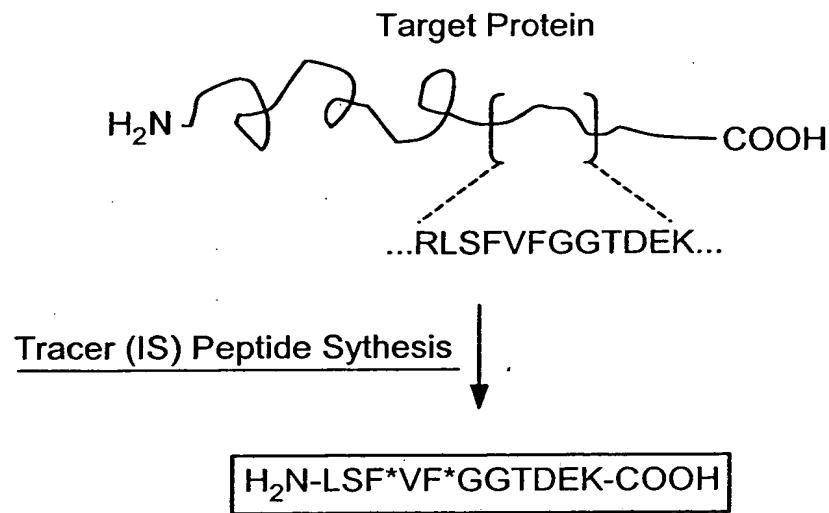


FIG. 1

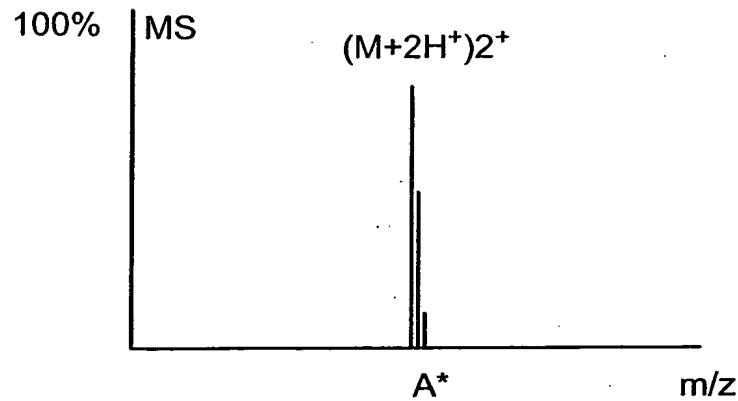


FIG. 2

BEST AVAILABLE COPY

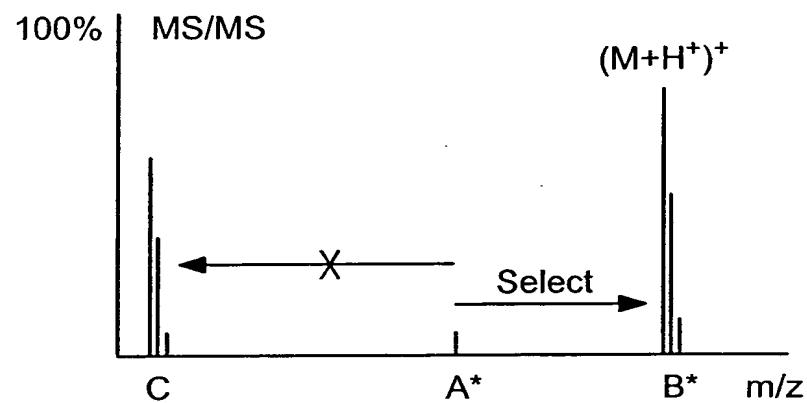


FIG. 3A

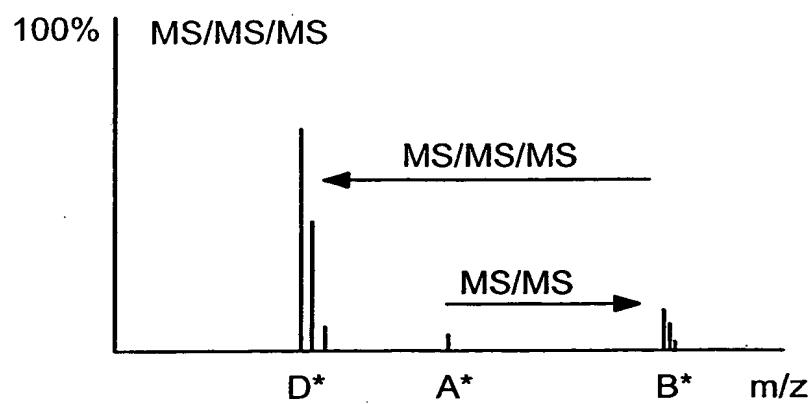


FIG. 3B

10 / 506877

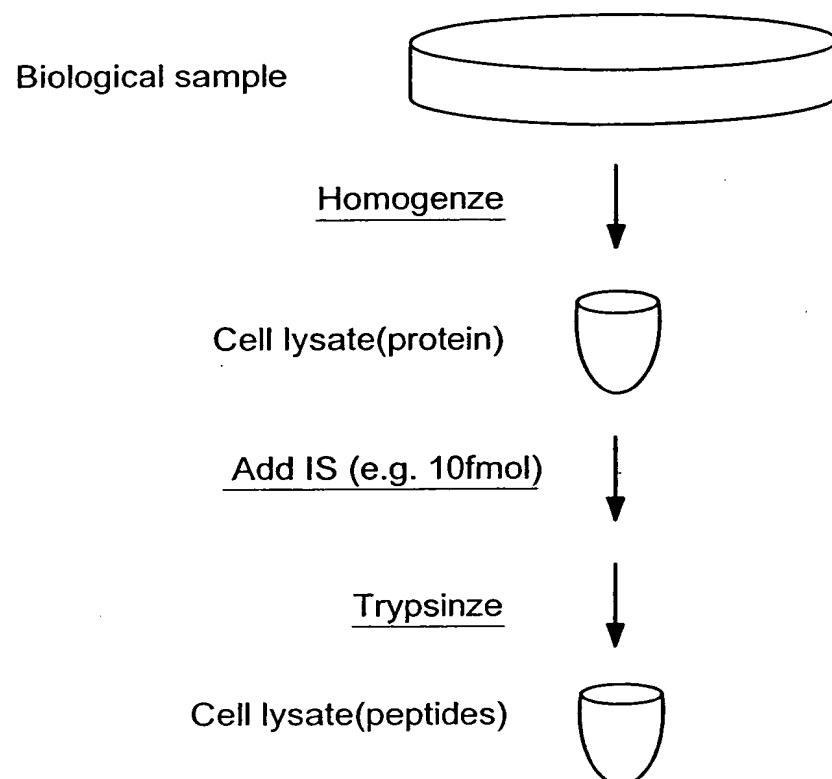


FIG. 4A

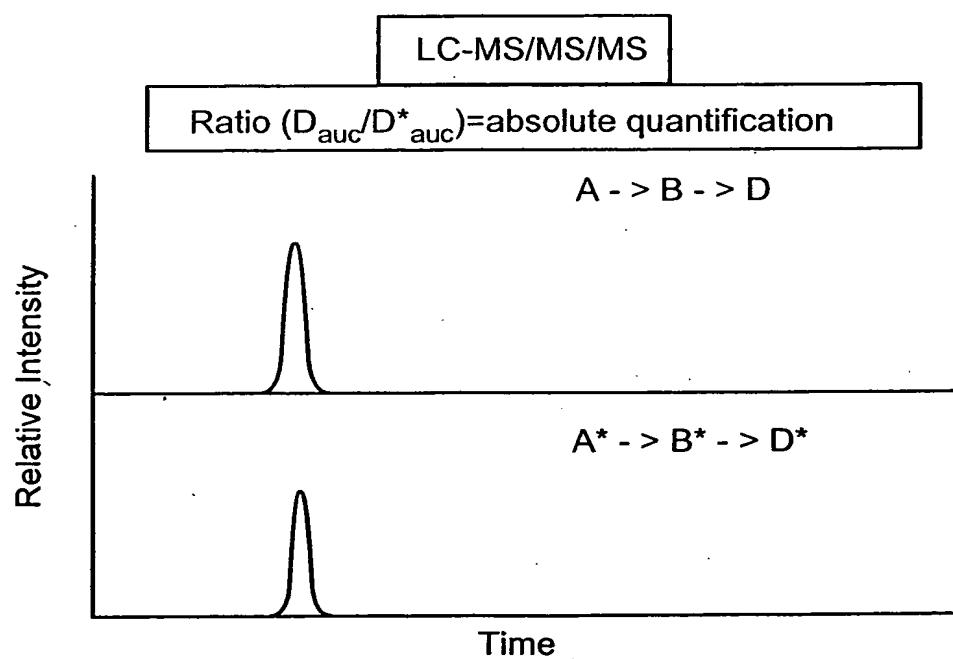
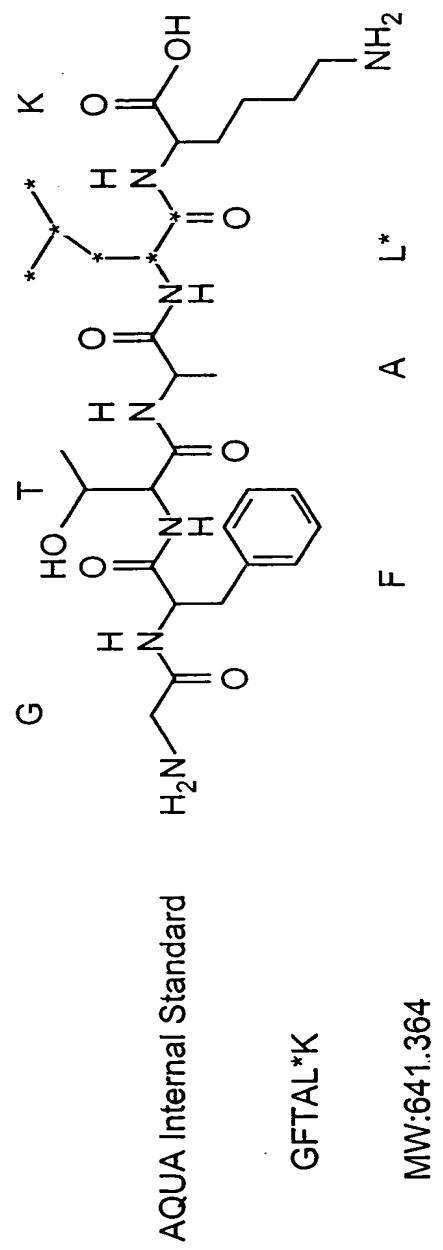
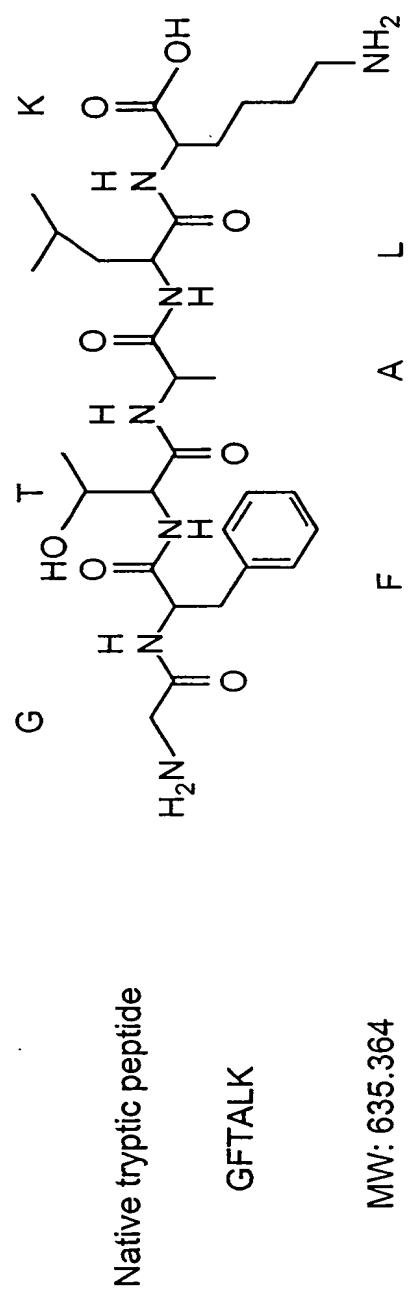


FIG. 4B

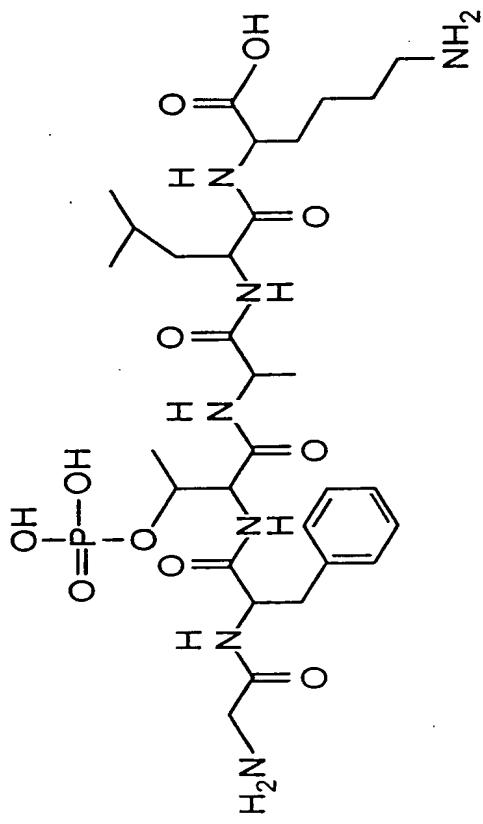
101506877



\* = stable isotope (e.g. <sup>13</sup>C)

FIG. 5A

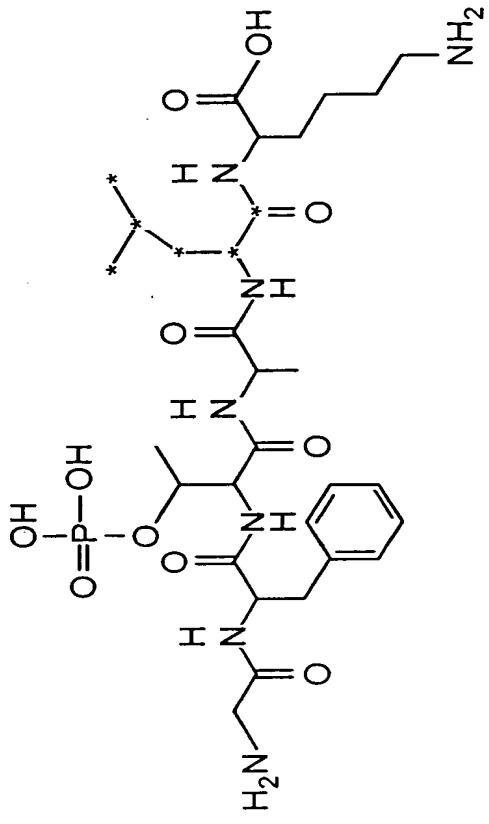
101506877



Native phosphopeptide

GF(pT)ALK

MW: 715.754



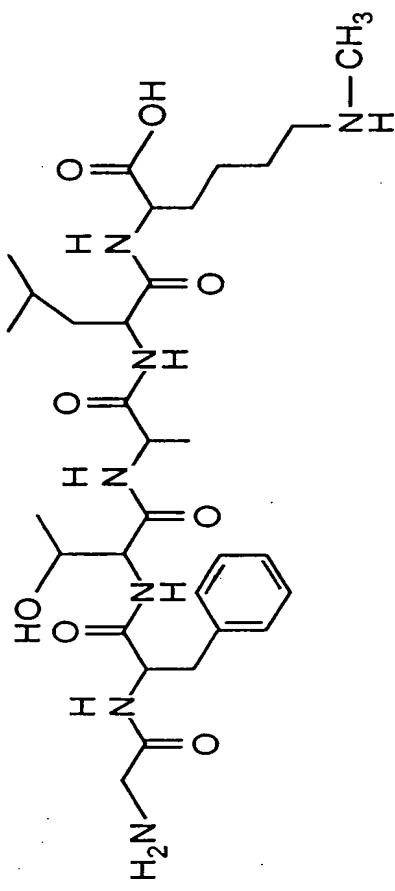
AQUA phosphopeptide  
Internal Standard

GF(pT)AL\*K

MW: 721.75

\* = stable isotope (e.g.  $^{13}\text{C}$ )

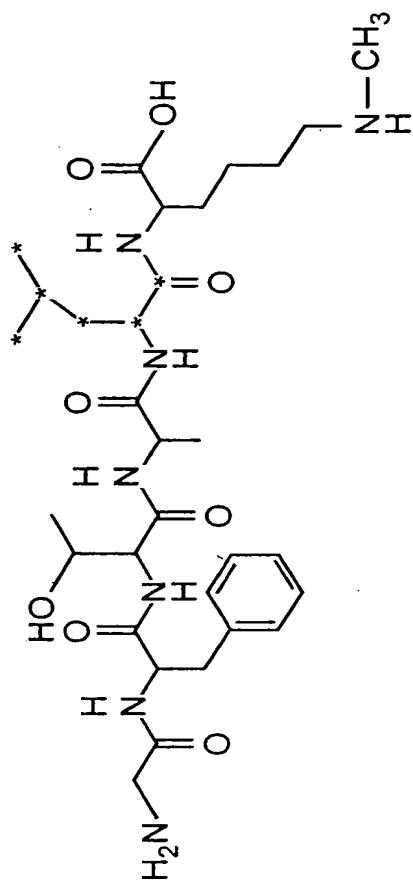
FIG. 5B



Native methylated peptide

GFTAL(mK)

MW: 649.79

AQUA methylated peptide  
Internal Standard

GFTAL\*(mK)

MW: 655.79

\* = stable isotope (e.g. <sup>13</sup>C)

FIG. 5C

10/506877

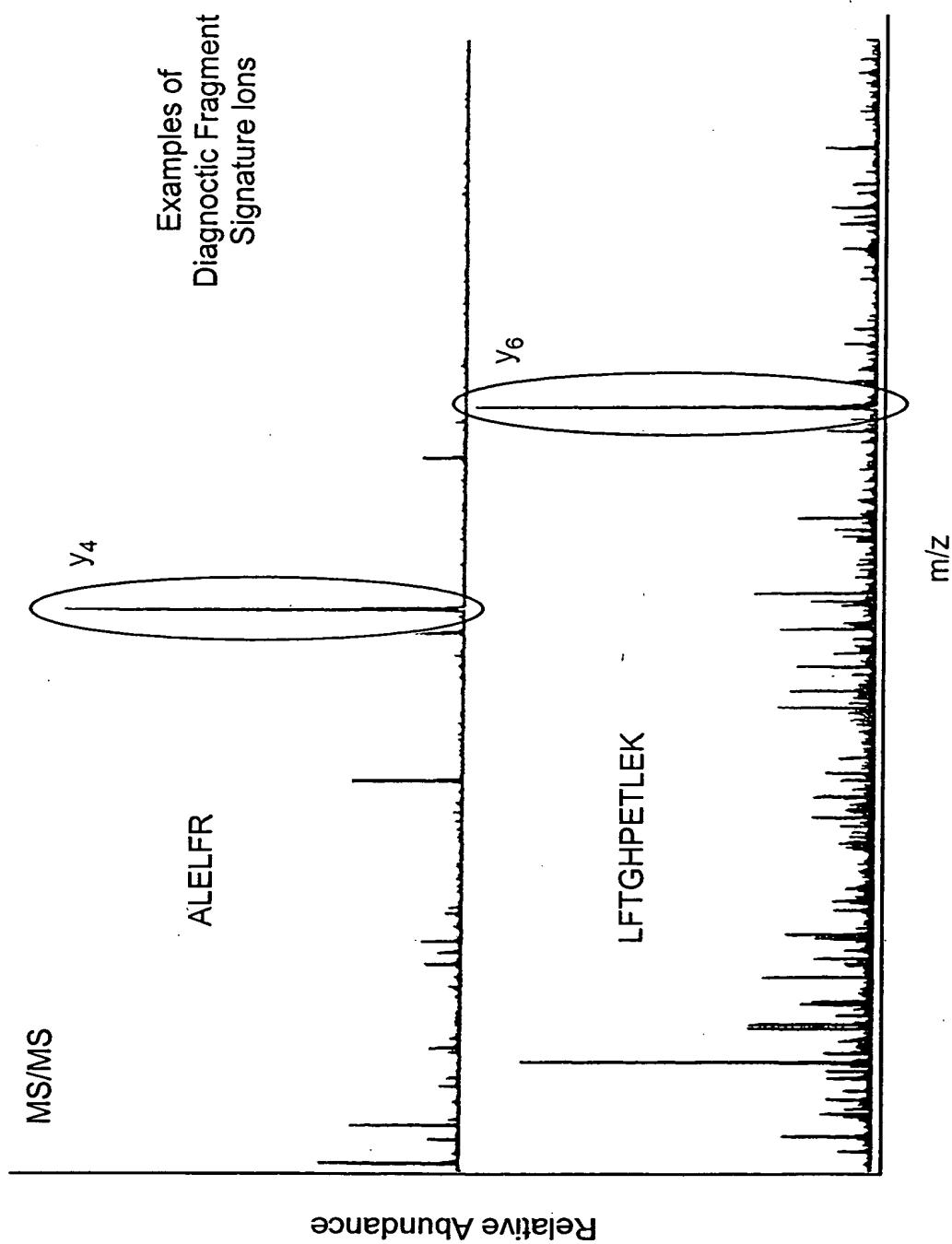


FIG. 6

10/506877

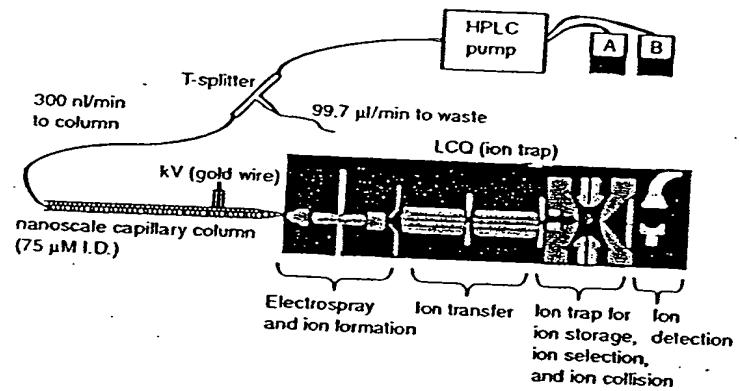
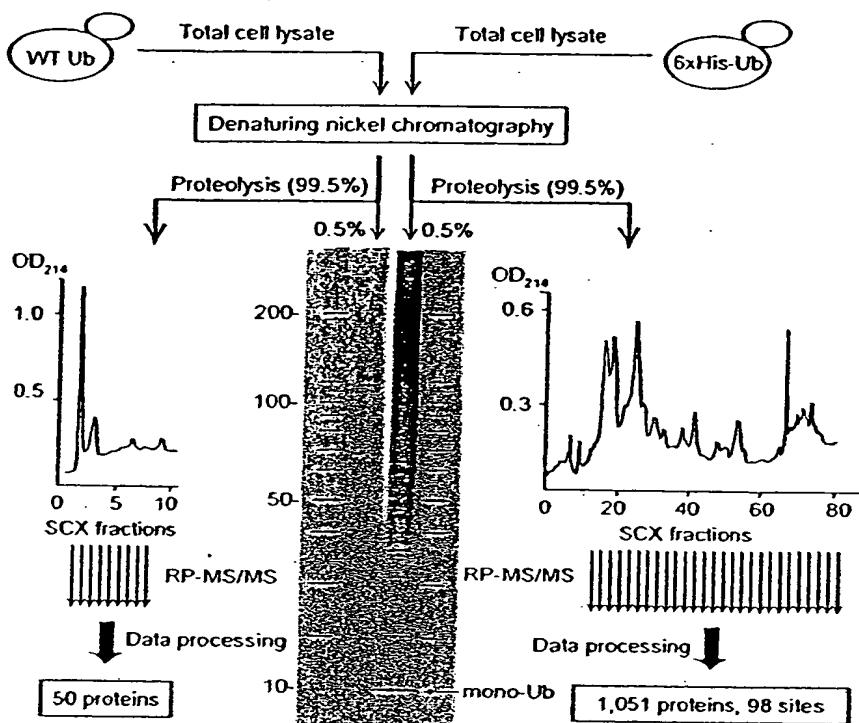


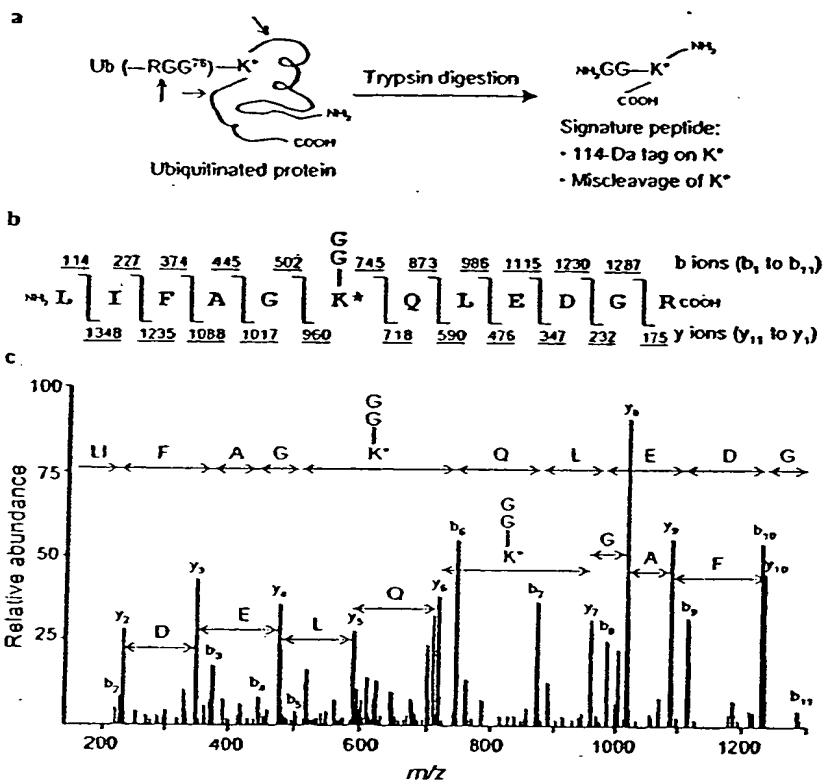
Figure 7

Figure 8



10/506877

# Figures 9A-9C



10/506877

# Figures 10A-10C

a

Name	#sites	Sequence
Ubiquitin	5	MPI TSPVVA KNSHESL DLSKQPF QSPPTQDGS NQRRRSSTI RQALSSLLGG ANVHS PAVLN NTIKCGNNG NI RSSNTDAQ LLGKQKQGP PPNARRHSTI AY QCSIS DSA
ECM21	5	TTTPRSSTSD TNARTSRLS YDQEPRI SGQ RYSQIEEDST YLD FDDHNS SAVVSSDLSS
SAM2	4	TSLTLRANS KFNEQFLI EY LTARGLQPK TVLSNEYLQ SI STSGESVF LPTI SSNDIE
YHR097C	3	YLSRLRNLND GTDAAEADFF MDG DQEGN TPSLATIAMA TESCGSI NEN RDILLRENN GHP CGSEL NTRSEI DGS MYSYI AVI V SVQGPTRTD MQLEUCSRVK VFWNTGVPP
HXT7	3	KTNEEFLYA ASMVKNLDE NDLFVPLS SPDDQM ENN SNDRQRFLK N PTEERLYL
GNP1	3	DKTAKASLL NAI DYNKTHL YQFCQYFLV PVVFSNHPE TI YLPSARYS YRRLATKA NRKGFYRQDS NSPQPI VSPD SSSSLSSSTS SLXLTETESA QAHRI SNTL FSXVQHILHM
YIL041W	2	SSHQHNEES GEEDIAEYZE YXVI RTPPRV AVSTANXPI Y JNRVWTDLS YEISFAQKV SLSNEVPI KI XLAJI QOVC VRX HVSITE RTVTFVSKGE YEVDQDIPVA XQFYNPYILD
YHL010C	2	FASXRRKERS VSLFEI RTXE XGTRALREEN VENFSNDNLE SYSPFDQDQD SNCNPKERLG JTEPI I ETK LKFPKVEELD KRTAKI I PPY G DAYTSI PA PEHA VANGPS HRPVSYI QFL
LSB1	2	SOHKCISHE ENEXPYVYDPF FHQTJ ASNS GLP VITHTRI NTPKRLYLD SLHFSNVYOR HOLEI MRLS XPDPECPSSLX RMEYLI DTP JFLVSEQQS QNLPTYDM ATHEOKQV PLSMNSIFG NCOPPPPTF EAI SUPASPI VSPMCBNM ASYDPLLSI QQLNLS KITS
ZE01	2	VSPGSCYSD AGVPNVNROS I SHANAMNG I SNSAFVSGN SOGVARARA TSVNDRSRFN
PHO84	2	NLDKLSTPS PYNRSHNSSTI NOLSOANGT VRUPNATTEN SKDKQEFFX XGYTLANVMD
URA3	2	DEEQEI VSS SSAQSLLSHG NEPPRYDEI V PLMSDEE
GDH1	2	
ERG5	2	
ERG3	2	
YCR268C	2	
YMR295C	2	

b

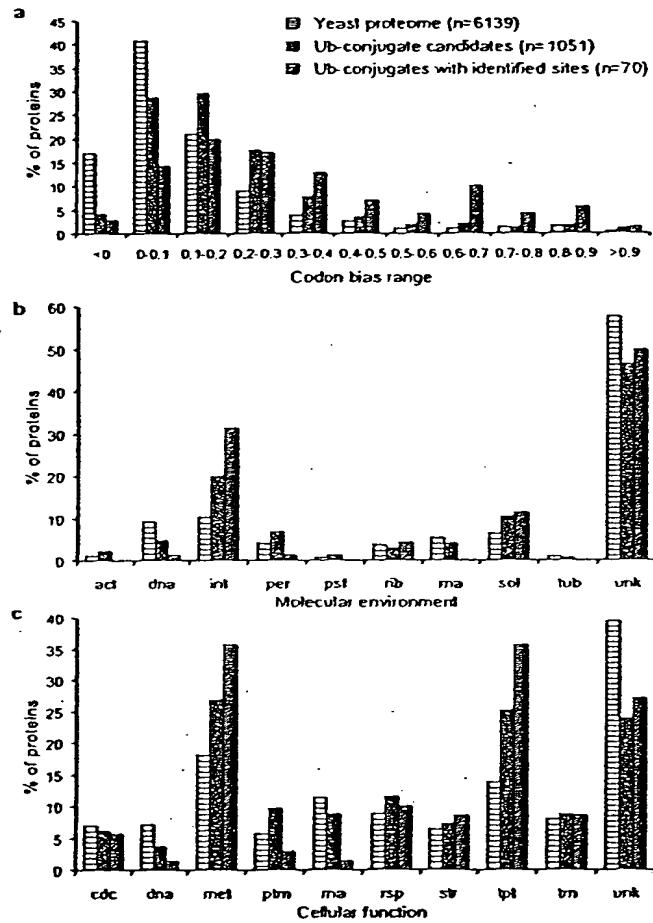
Site in Ub (K)	Signature peptide	SCX fraction	Abundance
48	LIFAGK*QLEDGR	48-56 (9)	high
63	TLSVDNIQK*ESTLHLVLR	65-72 (8)	high
11	TLTGK*TITLEVESSDTIDNVK	38-42 (5)	medium
27	TITLEVESSDTIDNVK*SK	41 (1)	low
6	LISEEDLGMOIFVK*TLTGK	38 (1)	low

c

Name	Phosphopeptide sequence	Description of function
ACC1	AVS*VSDLSSVANSOSSPLR	Acetyl-CoA carboxylase
CCC1	GSGGTSELGGSEST*PLLRL	Protein potentially involved in calcium regulation
CH01	DENDGYAS*DEVGGTLSR	Phosphatidylserine synthase
CHS1	DDEYDDLNT*IDK	Chitin synthase I
CHS3	NPSTLLPTIS*SMFWNK	Chitin synthase III
ECM21	NEES*GEEDIAEYPIK HALSS*LLGGANVHSPAVLNNTTK RPS*VIGFLSGHK S*HNSS*PTNGLSQAONGTVR	Protein possibly in cell wall biosynthesis
GCD6	EEIDS*EEFEDEDFEK	Translation initiation factor eIF2B
HSP30	ASGETAHEPEPEPAEQADEVDT*A	Heat shock protein located in cell membrane
LYP1	LOVVSNET*DINEEEEAHYEDK	High affinity lysine-specific permease
MET4	KYS*DNEEDDEYDDADLHGFKEK	Transcriptional activator
MYO3	RGS*VYHVPLNPVQATAVR	Myosin type I
PHO84	IHDTS*DEDDMANGLER	Inorganic phosphate/H <sup>+</sup> symporter
RAD16	NNDISSSS*PSQLOHEA SVNNYNELS*DDDTAVK	Nucleotide excision repair protein
Ubiquitin	TL*SDNIQK	Protein for posttranslational modification
YDR119W	IEEINENS*PLLSAPSX	Member of major facilitator superfamily (MFS)
YDR348C	TNS*FDMPQNLTR	Protein of unknown function
YHR097C	ETVDDDSET*LNOLODR LPSEEEAAGT*PK	Protein of unknown function
YOR042W	KNPDEDEFLNS*DDEM SSGIDEDEVTT*PAEADAKEEEHHPLPAR	Protein of unknown function
	E0HEDS*EEEDSWSOFVEK	
YPL019C	HYADLEDHES*S*DEEGTALPK	Vacuolar polyphosphate accumulation

a, Proteins identified containing multiple ubiquitination sites. b, Five lysine residues in ubiquitin were found to be sites for (poly)ubiquitination. The number in parenthesis indicates the total fractions in which the signature peptide was detected. c, List of phosphorylated peptides from the Ub-conjugates candidates. Phosphorylated residues are indicated by asterisk. Three peptides contained multiple (two) phosphorylation sites. There were 19 proteins identified from 26 phosphopeptides with 29 total phosphorylation sites. Of special interest is the phosphorylation of S<sup>37</sup> in Ub itself. The sequence of ECM21p is also shown with 5 ubiquitination and 5 phosphorylation sites highlighted. Sixty five other unmodified peptides were also identified from this protein (not shown for clarity).

# Figures 11A-11C



Comparison of protein expression, environment, and function among the yeast proteome, 1,051 candidate Ub-conjugates, and 70 proteins with detected Ub sites. **a**, Codon bias value (an indicator of protein expression levels with a value < 0.1 indicating likely low-copy-number proteins). **b**, protein molecular environment. act: actin-associated; dna: DNA-associated; int: integral membrane; per: peripheral membrane; psf: protein synthesis factor; rib: ribosome-associated; sol: soluble; tub: tubulin-associated; rna: RNA-associated; unk: unknown. **c**, cellular function. cdc: cell division control; dna: DNA recombination, replication and repair; met: metabolism; ptm: protein posttranslational modification and degradation; rna: RNA transcription, processing and turnover; rsp: responses to environment; str: cellular structure; tpt: transport of proteins and small molecules; trn: protein translation and assembly; unk: others and unknown.

**This Page is Inserted by IFW Indexing and Scanning  
Operations and is not part of the Official Record**

**BEST AVAILABLE IMAGES**

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

- BLACK BORDERS**
- IMAGE CUT OFF AT TOP, BOTTOM OR SIDES**
- FADED TEXT OR DRAWING**
- BLURRED OR ILLEGIBLE TEXT OR DRAWING**
- SKEWED/SLANTED IMAGES**
- COLOR OR BLACK AND WHITE PHOTOGRAPHS**
- GRAY SCALE DOCUMENTS**
- LINES OR MARKS ON ORIGINAL DOCUMENT**
- REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY**
- OTHER:** \_\_\_\_\_

**IMAGES ARE BEST AVAILABLE COPY.**

**As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.**